PB-228 807

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Environmental Health Service
Rockville, Maryland

1974

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The Persistence of Lignin in River Waters

R. C. Kroner and W. Allan Moore

Because of the tremendous volume of wastes discharged from paper and pulping operations, lignin ranks high as an industrial contaminant of surface waters. However, little is known concerning its decomposition in natural biological processes. This paucity of information is even more pronounced with regard to the biological decomposition of industrial forms of lignin in streams receiving these wastes.

Although the structure of lignin is not clearly understood, certain basic facts are usually accepted. Lignin from different sources is known to vary in chemical structure, although certain structural properties are common to all kinds of lignin. For instance, while lignin from different types of wood are known to contain methoxy and hydroxyl groups, the numbers of these groups seem to vary[8,9]. The problem is further complicated by the fact that methods employed in the isolation of lignin probably alter the original structure[4,8,9].

The Determination of Lignin

For the reasons just mentioned, no entirely satisfactory method is available for the estimation of lignin, expecially in microquantities. For purposes of this study, the method required should be able to give reasonably accurate results in the range from about 1.0 to 10.0 p.p.m., inasmuch as streams receiving lignin wastes could be expected to carry this amount.

As a starting point for the estimation of lignin in microquantities, two methods were chosen for examination:

1. A spectroscopic method utilising absorptive properties in the ultraviolet range of the spectrum, and,
2. A chemical method, utilising properties of structure which would result in a colorimetric reaction.

(1) Determination of Lignin by Ultraviolet Absorption

Absorption curves obtained on lignin samples from different sources exhibit a characteristic pattern of absorption in agreement with other investigators[2]. Maximum absorption occurs in the lower ultraviolet range with a characteristic plateau occurring in the range from 260 to 280 millimicrons (Figure 1). Since maximum accuracy is not obtained from readings taken in the shorter wave length range, 270 millimicrons was chosen as the arbitrary point for absorption readings. This point yields readings covering the range from 0.1 to 7.0 p.p.m. of lignin (Figure 2). The results obtained are in close agreement with those obtained in the subsequent colorimetric procedure (Table III).

Where settled river water was dosed with lignin, initial measurements using the ultraviolet and colorimetric procedures gave good agreement. As the solution aged, lignin measured by the latter method decreased. Measurements made in the ultraviolet, however, showed an apparent increase in the concentration of lignin. At the same time it was found that an unidentified

green algae growing in distilled water was responsible for an absorption reading at 270 millimicrons. It was therefore concluded that the by-products of biological activity, as well as the organic degradation products of lignin (such as furfurals and sugars) gave interfering absorption readings in the ultraviolet region. Results from the ultraviolet measurements are therefore not included in this paper.

(2) Determination of Lignin by Folin-Denis Procedure

Since lignin is known to be aromatic in structure[10], and contains several hydroxyl radicals[3,10,11], it was thought that the methods applicable to the determination of phenolic type compounds might be used for its determination. Accordingly, those methods, employing diazotized p-nitroaniline, Milon's reagent, Gibbs reagent, p-aminodimethylaniline and the more recent 4-aminoantipyrine were systematically examined.

In general, these methods produce a colored reaction product with lignin but are lacking in sensitivity and stability. The 4-aminoantipyrine method for phenols as outlined by Ettinger and Lishka[6], showed some promise except that the surface active properties of lignin in NaOH solution made the required chloroform extraction difficult to perform.

The most satisfactory method investigated was that of Folin and Denis[5], using phosphotungstic-phosphomolybdic acid reagent. The method has also been used by Mehta[12] for the determination of lignin and is outlined in Standard Methods for the Examination of Water and Sewage, 9th Edition. This method, slightly modified, was used in the present study.

Folin-Denis reagent reacts with lignin to form a blue reduction product of tungstic oxide[13] in the presence of sodium carbonate. Other organic compounds such as aromatic amines, phenols and related compounds, urea, etc., also form colored reaction products with Folin-Denis reagent. However, these compounds would normally be present in river waters in quantities so small as to offer only minor interference.

For the colorimetric estimation of lignin, 1.0 gram of the compound (commercial sample percent purity unknown) is dissolved in 1.0 liter of approximately 0.002 N NaOH to make a 1000 p.p.m. stock solution. The stock solution is then set up in a series of samples of 100 ml. volume ranging from 0.5 to 10 p.p.m., using distilled water as the diluent. Two ml. of the Folin-Denis reagent are added, followed by 5.0 ml. of saturated sodium carbonate solution. After a reaction time of two hours, the resulting blue colors are read photometrically against a similarly prepared blank. The relationship between the amount of color and the concentration of lignin follows Beers law from about 0.5 up to 8.0 p.p.m. (Figure 3).

For the determination of lignin in clear, settled river water, the same relationship is observed. However, a smaller amount of color per unit of lignin is obtained when the reaction is carried out in turbid river water. Clarification of turbid samples containing lignin both prior to and after reaction with Folin-Denis reagent does not serve to facilitate maximum color development. Experiments in which powdered clay was added to tap water as artificial turbidity confirms these results (Table 1).

The loss of color when the reaction is carried out in turbid samples is attributed to the adsorption of lignin or its colored reaction product by the suspended particles present. The same result, but more exaggerated, is obtained when the experiment is repeated using activated carbon.

It was further demonstrated that the difference in salt content of natural surface waters had little effect on color formation (Table II).

This procedure was, therefore, considered suitable for persistence studies of lignin in surface waters, provided that suitable precautions were taken to remove interfering turbidity.

Persistence of Lignin in Natural Waters

A survey of the literature reveals that comparatively little work has been done on the decomposition of lignin and its industrial forms in surface waters. Adams and Ledingham[1] studied the decomposition of synthetic lignosulfonate by wood and soil destroying fungi and reported that lignin was effectively utilized by only two species out of the 106 cultures investigated. This work was done in a moist culture medium and not in dilute solution. Zobell and Stadler[15] found that lignin was utilized by bacteria occurring in water from a number of different lakes and ponds. Again, solid media was used to determine lignin utilization. In a study of lake muds, Erikson[7] isolated strains of actinomycetes that were active in the decomposition of lignin. Other investigators[14] have isolated the same strains of organisms

from a variety of waters but the effect of the organisms on lignin decomposition has been studied only to a limited extent.

In general, literature on the decomposition of lignin indicates:

1. The species of microorganisms that utilize lignin are relatively limited.
2. Biological decomposition is very slow and incomplete.
3. The susceptibility of lignin to biological attack varies with its source, age, treatment, chemical nature, etc.
4. Higher organisms such as actinomycetes and fungi are more capable of utilising lignin than are bacteria and simple microorganisms.

Persistence of Lignin in River Waters

The conditions under which the experiments were made are shown in Table 4. The experiments were continued for periods ranging from 15 to 22 weeks and samples were withdrawn for lignin analyses periodically. The pertinent results are also presented in Table 4.

In Experiments A, B, and C, the overall persistence was greater than five months. About 60 percent of the pine lignin originally added still remained after 22 weeks of observation under both aerobic and anaerobic conditions. In Experiment C, with the lignin-containing water exposed to strong sunlight at room temperature, only 24 percent of the pine lignin remained after 20 weeks. Algae growths, completely absent in Experiments A and B, appeared in Experiment C after three weeks and continued to increase slowly even after 16 weeks.

From observation of the results, it is indicated that algae resulting from sunlight and favorable temperatures are instrumental in hastening the dissimulation of lignin by microorganisms. In Experiments A and C, the substrates and dosages were similar, the variables being light, degree of aeration, and slight variation in temperature. The rate of dissimulation in Experiment C in indirect sunlight was nearly three times as fast as in Experiment A.

In experiments C and D, all conditions were similar, the only variable being the source of lignin. In Experiment C, the water was dosed with 10 p.p.m. pine lignin dissolved in 0.002 N NaOH, while in Experiment D, the water was dosed with sulfate waste liquor from a Kraft process mill in which the lignin is present in a modified state. The persistence data indicate that in both studies the lignin was converted at similar rates.

In Studies D and E, the variable consisted of difference in source of substrate. The substrate used in Experiment D was settled Ohio River water with no positive history of lignin pollution. This substrate was innoculated with sulfate waste liquor from a process mill in Louisiana. In Experiment E, the substrate was settled impoundment water from a TVA reservoir with a positive history of sulfate waste liquor and sewage pollution. This sample was dosed with sulfate waste liquor from a Kraft process mill located on the same stream from which the substrate was taken. Observations on the lignin persistence in these studies over a twelve week period showed a slightly greater assimilation rate in Experiment E than in Experiment D. Visual signs of biological activity were evident three days after innoculation with the

sulfate waste liquor in Experiment E. Similar signs of activity did not appear in Experiment D until a lapse of approximately two weeks. However, the previous history of lignin pollution in Experiment E did not produce a pronounced faster rate of assimulation than Experiment D, which was free of any previous lignin pollution.

In each experiment the initial pH of this substrate (7.0 to 7.8) was maintained throughout the observation period. Very little pH control was required, however. Throughout these persistence studies the analytical data was somewhat erratic. These variations are probably due to a stepwise decomposition into simpler compounds. These degradation products also react with the Folin-Denis reagent, giving discordant results. However, these simpler compounds are further broken down by other microorganisms present in the system. The net result is the establishment of an overall trend in the disappearance of lignin from the system under consideration.

Summary

In summary, the results indicate that:

(1) The rate of reduction of lignin at 20°C. in the absence of light was the same under aerobic and anaerobic conditions.
(2) Aerobic exposure to sunlight at room temperature resulted in a greater reduction of lignin.
(3) Lignin was removed more rapidly from water in which algae grew.
(4) Substrate water from a source polluted with sewage and sulfate waste liquor did not materially affect the rate of lignin decomposition.

Table I

Effect of Turbidity on Color Formation of Lignin

with Folin-Denis Reagent

Sample Treatment	Added p.p.m.	Observed p.p.m.	Per cent Recovery
A. Distilled Water Substrate No turbidity added	5.0 5.0	5.0 5.0	100.0 100.0
B. Distilled Water Substrate 1000 p.p.m. of dried powdered clay added 20 hours prior to test. Clarified and filtered prior to reading.	5.0 5.0	4.58 4.51	91.6 90.0
C. Distilled Water Substrate Test carried out on clear sample, then 100 p.p.m. dried powdered clay added. Sample clarified and filtered prior to reading.	5.0 5.0	4.82 4.74	96.3 94.8

Table II

Effect of Dissolved Solids on Color Formation of Lignin with Folin-Denis Reagent

Sample Source	Dissolved Solids p.p.m.	Lignin added p.p.m.	Per cent Recovery
Distilled water	0	5.0	100.
Ohio River	250	5.0	100.
Britton, South Dakota	2600	5.0	98.5
Cocoa, Florida	455	5.0	99.6

Table III

Determination of Lignin Using Colorimetric

and Ultraviolet Methods

Sample	Lignin Content* p.p.m.	Lignin Found Ultraviolet 270 mμ 10.0 cm. Cell	Lignin Found Colorimetric 670 mμ 5.0 cm. Cell
1	0.50	0.58	0.60
2	6.0	6.1	6.3
3	8.0	8.4	8.3
4	13.5	13.1	15.0
5	22.5	20.1	23.6

* Samples prepared from commercial lignin dissolved in .002 NaOH.

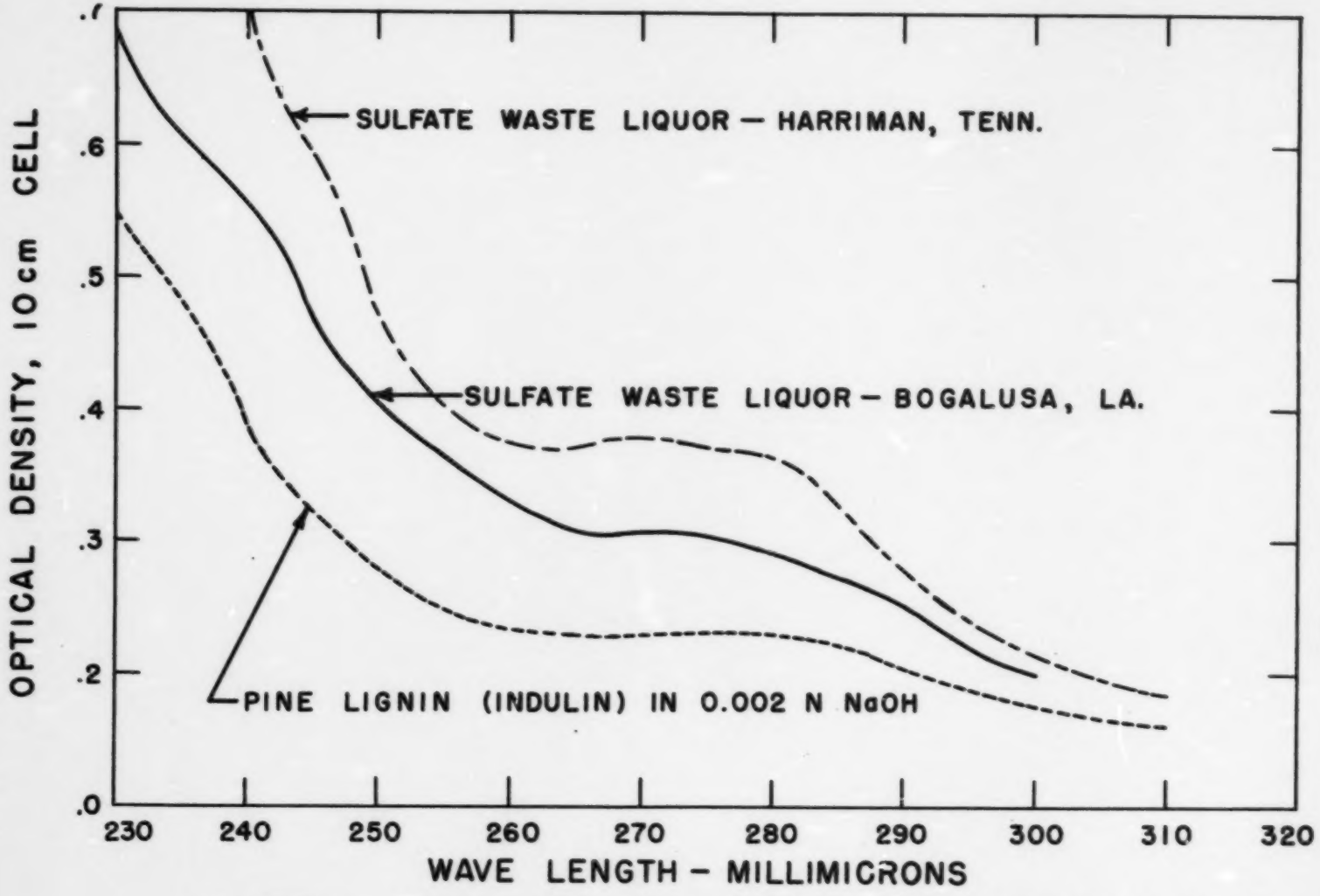
Table IV

Persistence of Low Concentrations of Lignin in Water Under Various Conditions[1]

Experiment	A	B	C	D	E
Substrate	Ohio R. Water	Ohio R. Water	Ohio R. Water	Ohio R. Water	TWA Impoundment Water
Temperature During Experiment	20°C.	20°C.	Room 25-30°C.	Room 25-30°C.	Room 25-30°C.
Condition of Aeration	Aerobic, Bubbling air	Anaerobic[2]	Aerobic, Daily shaking	Aerobic, Daily shaking	Aerobic, Daily shaking
Light Exposure	Dark	Dark	Indirect Sunlight	Indirect Sunlight	Indirect Sunlight
Source of Lignin	Pine Lignin[3]	Pine Lignin[3]	Pine Lignin[3]	Sulfate[4] Waste Liquor	Sulfate[5] Waste Liquor
Lignin Remaining ppm					
Initial	10.1	8.7	10.0	6.8	9.1
4 Weeks	9.6	7.9	8.3	5.1	6.3
8 Weeks	9.0	7.6	7.2	4.6	5.2
12 Weeks	8.5	7.0	5.7	3.9	4.8
16 Weeks	7.3	6.3	3.8	3.1	3.7
20 Weeks	6.4	5.8	2.4	-	-
Percent Lignin remaining at end of Expt.	64.	67.	24.	46.	41.

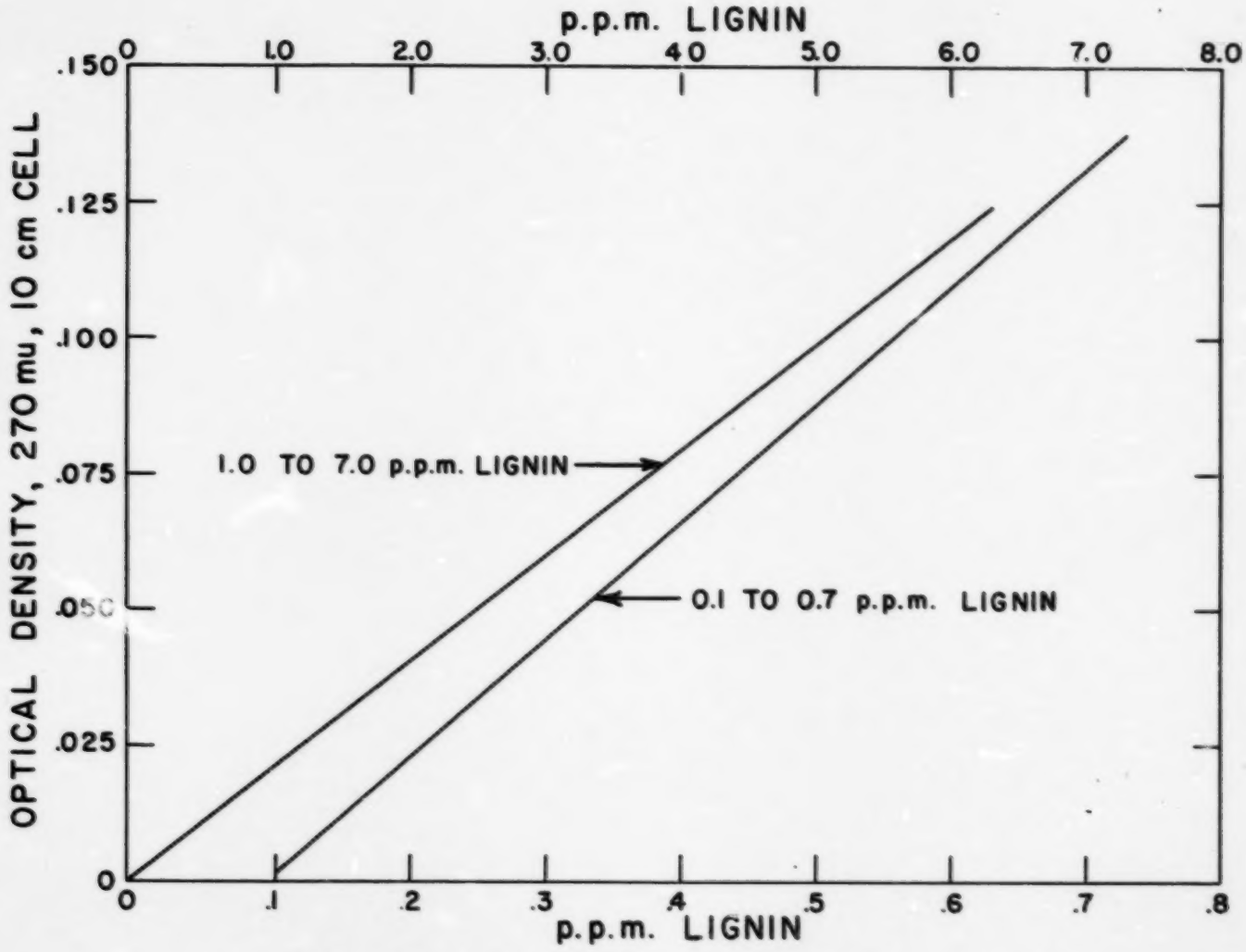
(1) pH maintained at 7.2
(2) Septic sewage dilution maintained by exclusion of oxygen with an alkaline pyrogallol train
(3) Commercial sample (Indulin) dissolved in .002 N NaOH
(4) From Kraft Process, Bogalousa, La.
(5) From Kraft Process, Harriman, Tenn.

12



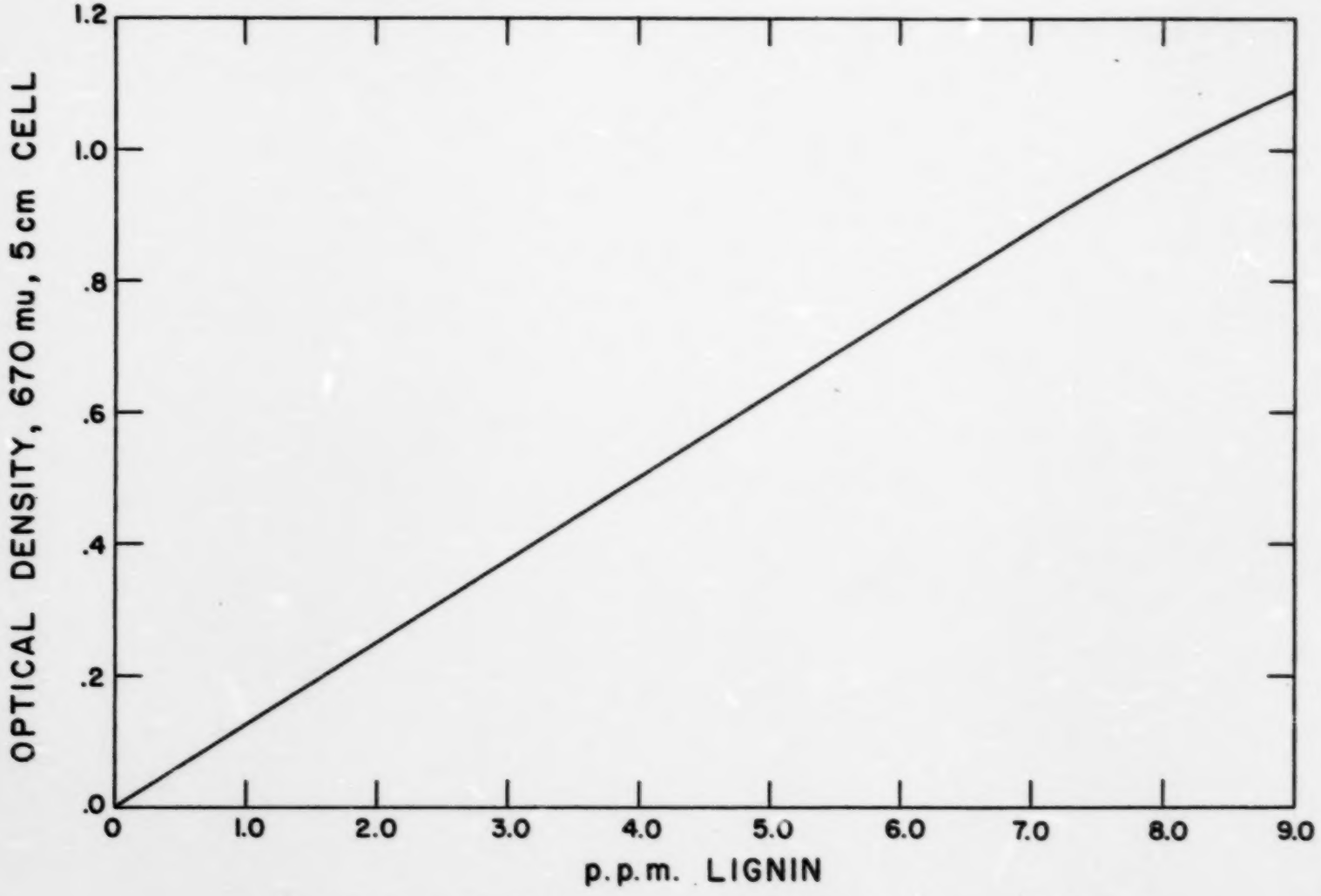
ULTRA VIOLET ABSORPTION CURVES FOR LIGNIN FROM DIFFERENT SOURCES

FIGURE 1



ULTRA VIOLET STANDARD CURVE
FOR LIGNIN IN DISTILLED WATER

FIGURE 2



LIGNIN WITH FOLIN – DENIS REAGENT

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FIGURE 3

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